BARD Research Grant
Final Scientific Report

Cover Page

BARD Project Number:
IS-4125-08C
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Project Title:
The molecular and biochemical basis of terpenoid aroma formation in tomato

Investigators	**Institutions**
Principal Investigator (PI):	
Efraim Lewinsohn,	ARO, Israel
Co-Principal Investigator (Co-PI):	
Natalia Dudareva	Purdue University
Collaborating Investigators:	
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Yaron Sitrit	Ben Gurion University

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Flavor, metabolic engineering, geraniol, zingiberene

Abbreviations commonly used in the report, in alphabetical order**:**

Budget: IS: $ 150.000 US: $ 155,000 Total: $ 305,000

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1		0	1
Submitted, in review, in preparation	3			3
Invited review papers				
Book chapters			1	1
Books				
Master theses				
Ph.D. theses			1 in preparation	1
Abstracts	5	1	5	11
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	2		3	5
Longer Visits (Sabbaticals)				

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

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Abstract

The original objectives were defined as follows:

1. Discovery of genes that affect the unique aroma of tomato.

Varieties displaying superior "original" aromas as well as key introgression lines (*L. esculentum* x *L. pinpinellifolium*), kindly provided by Dr. I. Levin, Volcani Center, were organoleptically tested in search for the key chemicals and the genes that affect the formation of critical aroma compounds in tomato fruit. Volatile compositions and organoleptic properties of the tomatoes were evaluated. Our results indicated that none of the heirloom varieties, introgression lines or other tomato accesions tested consistently contained any particular volatile that might impart the key aroma to tomato. This avenue of research was therefore modified and we opted for searching genomic and transcriptomic databases of tomato for genes likely to affect the tomato aroma. Two novel carotenoid cleavage dioxygenases were identified and partially characterized for functionality, and their contribution to tomato aroma was assessed. This work was presented in several national and international meetings as posters .

2. Genetic engineering of the early terpenoid pathway in tomato fruit to produce elite varieties with tailor-made colors and aromas.

The diversion of the existing carotene biosynthetic pathways to the biosynthesis of flavor-influencing terpenes was performed in previous BARD funded research. In the present work we augmented these studies by overexpressing the snapdragon (*Antirrhinum majus*) geranyl diphosphate synthase small subunit (GPPS-SSU) gene in tomato under control of the fruit ripening specific polygalacturonase PG promoter to divert the metabolic flux from carotenoid formation towards GPP and monoterpene synthesis. The resulting transgenic tomato fruits emitted and accumulated geraniol, geranial, neral, citronellol and citronellal at the expense of reduced carotenoid contents indicating a redirection of flux towards GPP and monoterpenes. Furthermore, co-expression of both the *Ocimum basilicum* geraniol synthase (GES) with snapdragon GPPS-SSU led to a more than 3-fold increase in monoterpene formation in tomato fruits. The results have been summarized in a paper currently in review in the Plant Journal.

Additionally, mutants defective in carotenoid biosynthesis (yellow*r*) and therefore putatively higher levels of monoterpene precursors, were also crossed with GES-transgenic plants, but the resulting hybrids did not show any different phenotype in the volatiles generated as compared to the GES-transgenic parental line. The crosses were not examined further.

3. Understanding of the intricate molecular and biochemical regulation of mono- and sesquiterpene biosynthesis in tomato fruit.

The objective was to rationalize the mechanisms by which directed increases in one targeted sesquiterpene or monoterpene bring about unexpected increases in other mono- and sesquiterpenes in tomato fruit. For that, we co-expressed snapdragon GPPS-SSU with *Ocimum basilicum* α-zingiberene synthase (ZIS), a cytosolic sesquiterpene synthase known to also form monoterpenes from GPP. The resulting tomato fruits of the GPPS-SSUxZIS cross emitted and accumulated significantly increased amounts of ZIS-derived monoterpene products as compared to fruits overexpressing ZIS alone. This indicated that redirection of the metabolic flux towards GPP in plastids also increases the cytosolic pool of GPP available for monoterpene synthesis in this compartment via GPP export from plastids. These findings have also been submitted for publication in the Plant Journal.

Agricultural and/or economic impacts of the research findings, if known.

Public concern about the deterioration of flavor of tomatoes is an issue with important commercial consequences and the focus of modern breeding programs directed towards quality and consumer acceptance. The discoveries described here open a route for biotechnological and conventional breeding solutions. Moreover, since many of the mono- and sesquiterpenes studied and its derivatives also have antifungal and insecticidal properties, the genes isolated and the fruits generated carry with them important agronomical repercussions in the area of plant protection.

Description of the Cooperation:

Transgenes for GES and ZIS that were generated in Newe Yaar were transferred to Purdue for further studies. Additional transgenic plants bearing the GPPS small subunit

were generated at Purdue and crossed with the plants provided. Phenotypic assessment and characterization of the volatiles generated were performed at Purdue. A search for an aromatic tomato was performed in the BGU lab using a variety of heirloom cultivars and introgression lines with *L. pimpinellifolium*. Other crosses of the *r* mutant with GES and ZIS tomato transgenes were also performed at Purdue. Bioinformatic analyses and isolation of two novel CCD's and their characterization were performed in BGU and Newe Yaar. The laboratory at University of Michigan aided in the cloning of constructs for the tomato transformation experiments.

EP visited Israel at least 4 times during the period of the grant. ND visited Israel to attend an International meeting. Additionally, all PI's met several times with each other in international meetings. Although most of the trips were not funded by BARD, they allowed the exchange of information and planning of the research. All partners communicate on a regular basis by phone, Skype and email. Undoubtedly, this project allowed for a collaboration without which this research may not have been accomplished by any single lab.

List of Publications:

Papers in Peer-Reviewed Journals:

Pichersky, E. and Lewinsohn, E. (2011). Convergent evolution in plant specialized metabolism. Annual Review of Plant Biology 62: 549-566.

Gutensohn, M., Orlova, I., Nguyen, T., Davidovich-Rikanati, R., Sitrit, Y., Lewinsohn, E., Pichersky, E. and Dudareva, N. (2012). Cytosolic monoterpene biosynthesis is supported by plastid-generated geranyl diphosphate substrate in transgenic tomato fruits. The Plant Journal (*Submitted*).

Leiderman, M., Davidovich-Rikanati, R., Bar, E., Dudareva, N., Pichersky. E., Lewinsohn, E., Ben-Shabat, S. and Sitrit, Y. (2012). Two novel carotenoid cleavage dioxygenases 4 in tomato (*Solanum lycopersicum* L.). Plant Science (*In Preparation*).

Leiderman, M., Davidovich-Rikanati, R., Bar, E., Dudareva, N., Pichersky. E., Lewinsohn, E., Ben-Shabat, S. and Sitrit, Y. (2012). Temporal differences in fragrance chemistry and within flower organs of tomato (*Solanum lycopersicum* L.). Genetic Resources and Crop Evolution (*In Preparation*).

Book Chapter

Davidovich-Rikanati, R., Leiderman, Tadmor., Sitrit, Y. and Lewinsohn, E. (2012). Tomato aroma: biochemistry and biotechnology, Recent Advances. In: "Biotechnology in Flavor Production" (2nd ed., eds. D. Havkin Frenkel, F. Belanger and N. Dudai). Blackwell Publishing. (In Preparation).

Abstracts and Presentations in International Meetings

Lewinsohn, E., Davidovich-Rikanati, R., Iijima, Y., Pichersky, E. and Sitrit, Y. (2009). Functional genomics for the discovery of genes affecting lemon basil aroma and their use in flavor engineering of tomato. *4th International Symposium on Breeding Research on Medicinal and Aromatic Plants (ISBMAP2009),* Ljubljana, Slovenia. (Oral Presentation).

Leiderman, M., Lewinsohn, E., Ben-Shabat, S. and Sitrit, Y. (2009). Two novel members of the CCD gene family in tomato (*Solanum lycopersicum* L.). *Metabolism, Metabolomics and Metabolic Engineering in Plants to Increase Crop Productivity and Nutritional Value, an International Symposium,* Ein Gedi, Israel. (Poster Presentation).

Lewinsohn, E. (2009). Metabolic engineering of tomato aroma: the sounds of silent metabolism. *SOL2009. The Sixth Solanaceae Genome Workshop,* New Delhi, India. (Oral Presentation).

Lewinsohn, E. (2010). Phytochemical diversity: The sounds of silent metabolism. *AROMED: International Symposium on Aromatic and Medicinal Plants.* Lucknow, India. (Oral Presentation).

Bar, E., Leiderman, M., Davidovich-Rikanati, R., Lewinsohn, E., Pichersky, E., Dudareva, N.**,** Ben-Shabat, S., Levin, I. and Sitrit Y. (2010). Heredity of fruit aroma volatiles in *Solanum lycopersicum* x *Solanum pimpinellifolium* interspecific crosses.*METABOLOMICS 2010. Breakthroughs in Plant, Microbial and Human Biology, Clinical and Nutritional Research, and Biomarker Discovery,* Amsterdam, The Netherlands. (Poster Presentation).

Leiderman, M., Lewinsohn, E., Davidovich-Rikanati, R., Bar, E., Ben-Shabat , S. and Sitrit, Y. (2011). Characterization of two novel members of the *CCD* gene family in tomato (*Solanum lycopersicum* L.). *The Annual Meeting of the Israeli Society of Plant Sciences 2011.* Sde Boker, Israel. (Poster Presentation).

Sitrit, Y., Leiderman, M., Ben-Shabat , S. and Lewinsohn, E. (2011). Novel carotenoid cleavage dioxygenases involved in the formation of volatile apocarotenoids in tomato (*Solanum lycopersicum* L.). *SOL-ICuGI 2011: 8th Solanaceae and 2nd Cucurbitaceae Genome Joint Conference,* Kobe, Japan. (Oral Presentation).

Gutensohn, M., Orlova, I., Nguyen, T., Pichersky, E., Lewinsohn, E. and Dudareva, N. (2011). Metabolic engineering of terpenoid production in tomato fruits. *Gordon Research Seminar, Plant Metabolic Engineering*, Waterville Valley, NH, USA (Poster Presentation).

Gutensohn, M., Orlova, I., Nguyen, T., Pichersky, E., Lewinsohn, E. and Dudareva, N. (2011). Metabolic engineering of terpenoid production in tomato fruits. *Gordon Research Conference, Plant Metabolic Engineering*, Waterville Valley, NH, USA (Poster Presentation).

Gutensohn, M., Orlova, I., Nguyen, T., Lewinsohn, E., Pichersky, E. and Dudareva, N. (2012). Metabolic engineering of cytosolic and plastidic monoterpene formation from GPP and NPP in tomato fruits. *3rd Banff Conference on Plant Metabolism*, Banff, Canada. (Poster Presentation).

Dudareva, N. (2012) Biosynthesis and metabolic engineering of terpenoid production in plants. *5th International Symposium "Breeding Research on Medicinal and Aromatic Plants"*, Vienna, Austria. (Oral Presentation).